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ORIGINAL ARTICLE

Associations of *Caspase-3* gene polymorphism with lumbar disc herniation

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Abstract To investigate *Caspase-3* gene polymorphisms (rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C) and susceptibility to lumbar intervertebral disc herniation (LDH). The genotype frequency distributions of the polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism in 107 LDH patients (case group) and 121 healthy individuals (control group). SHEsis software was used to conduct gene linkage disequilibrium and haplotype analysis. Regression analysis was used to analyze possible risk factors for LDH. Statistically significant differences in family history of LDH, amateur sports, leisure activities, bed types, and spine load grade were found between the case and control groups. The distribution of allele and genotype frequencies of rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C polymorphisms of *Caspase-3* were significantly different between the case and control groups. Haplotype analysis showed that the G-G-C (rs4647693-rs4647610-rs12108497) haplotype might be a risk factor for LDH, whereas the A-A-T haplotype might be a protective factor ($p < 0.05$). Binary logistic regression analysis showed that the GA+AA genotype of rs4647693 was negatively associated with the risk of LDH, whereas high spine load grade was positively associated with the risk of LDH. These findings revealed that rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C polymorphisms of *Caspase-3* may be associated with susceptibility to LDH and that interaction and modification effects may exist between *Caspase-3* polymorphisms.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Lumbar disc herniation (LDH), a type of chronic low back pain syndrome, is caused by the degeneration and herniation of the nucleus pulposus of the intervertebral disc [1]. LDH remains a considerable clinical problem that can affect up to 80% of individuals at any time in their lives, resulting in important social and economic consequences [2]. Specifically, LDH has an estimated worldwide incidence rate of 7.62% per year, with people aged 25—55 years at a higher risk of developing LDH [3]. Generally, LDH is considered a complex disease involving multifactorial interactions, but the precise etiology and pathogenesis underlying LDH is complex and still poorly understood. In the past few years, various factors such as sex, age, height, smoking habits, and physical activity have been reported in the literature as being associated with LDH [4—6]. In addition, recent molecular epidemiological studies have highlighted the potential and significant role of genetic variability in LDH [7—9]. To date, with the completion of high-quality sequencing of the human genome and a deeper understanding of disease mechanisms at the cellular and molecular level, the genes responsible for susceptibility to many complex diseases, including LDH, have been identified [4,10].

Caspase-3, also known as CPP32/Yama/apopain, is one of the most extensively studied apoptotic proteins in the cysteine—aspartic acid protease (caspase) family and is encoded by the *Caspase-3* gene [11]. Caspase-3 acts as the main executor of apoptosis and mediates both extrinsic and intrinsic cell death signaling pathways [12]. Apoptosis is considered an essential biological event, which is modulated by the activation of multiple caspases, including caspase-3, and plays a crucial role in cellular and tissue homeostasis. Thus, the inappropriate regulation of apoptosis might be one of the most important factors in influencing the development and progression of many diseases [13,14]. Therefore, it seemed biologically plausible that genetic alterations of *Caspase-3* might be involved in the failure of apoptosis, which might lead to human diseases such as LDH [15—17]. More importantly, there is evidence suggesting that the failure of apoptosis could reduce disc cell numbers and destroy normal disk function during disc aging and degeneration, thus resulting in diminished generation and organization, or ultimately leading to lumbar disc disease [18,19]. Thus, the *Caspase-3* gene, as an important apoptosis-related gene located at 4q35.1, is naturally a good candidate gene for LDH.

To test this hypothesis, the present study was designed to search for an association between *Caspase-3* single-nucleotide polymorphisms (SNPs) and haplotypes in LDH patients and to identify the mechanism of action of Caspase-3 in LDH.

Methods

Ethical statement

This study was approved by the ethics committee of Changsha Central Hospital, Changsha, China, and all participants signed informed consent forms before participating in the research. The ethical approval for this study conformed to the ethical principles for medical research involving humans of the Helsinki Declaration.

Study participants

From January 2011 to January 2015, 107 LDH patients in the spinal surgery department of Changsha Central Hospital and First Affiliated Hospital of Guangxi Medical University were enrolled in our experiment as the case group. All patients were from the Chinese Han population and included 68 men and 39 women, aged 19—58 years with a mean age of 38.75 $\pm$ 12.31 years. The diagnostic criteria were: (1) patients who had a history of lumbar sprain and/or a history of chronic strain; (2) patients who had pain in the inferior lumbar part of the spine and regional sciatic nerve pain in the leg caused by bed rest; (3) patients with tenderness beside the lumbar spine that affects the leg or foot; (4) patients whose lumbar flexion range was obviously limited; (5) patients with positive results in the straight-leg raising test and augmentation test (Bragard's sign); (6) patients who had the following nerve injury symptoms: muscular atrophy, motor weakness, decreased sensation and hyporeflexia; and (7) patients with clinical manifestations of LDH in accordance with imaging findings, including computed radiography, computed tomography, and/or magnetic resonance imaging. Exclusion criteria were: (1) patients with mental illness or severe dysfunction of the heart, lung, liver, or kidney; (2) patients with blood disease, diabetes, autoimmune disease, or tumors; and (3) patients identified as underweight/malnourished with a body mass index (BMI) < 18.5 kg/m^2 or overweight/obese with a BMI ≥ 28 kg/m^2. A total of 121 healthy individuals who received a physical examination in our hospital during the same period were allocated to the control group, including 71 men and 50 women, with an average age of 38.25 $\pm$ 11.73 years. Inclusion criteria of the control group were: (1) people of the Chinese Han ethnicity whose age and sex were matched to the patients in the case group; (2) good health as confirmed by physical examination; (3) no recent infections; (4) no history of tumors; and (5) history of lumbar sprain and/or chronic strain.

General information collected

(1) Spine load grade was based on the Swedish translated version of the Short Musculoskeletal Function Assessment Questionnaire (SMFA) [20], which is referred to as the core and occupational classification system [21]. The classifications are as follows: Grade I: freelance and less manual; Grade II: sedentary jobs; Grade III: mainly whole body vibration, bending over and twisting work; and Grade IV: lifting and heavy work. (2) Smoking was defined as having one or more cigarettes per day for 1 year or longer, or as smoking $>$ 18 packs of cigarettes per year. (3) Drinking was defined as having two or more drinks (of at least 100 mL) every week for 1 year or longer. (4) Amateur sports were defined as activities performed after-working hours such as household chores. (5) Leisure activities were defined as exercise performed three times a week for at least 20 minutes that led to an increased heart rate and sweating.

Strategies for tagging SNP selection

The International Hapmap Project (Han Chinese in Beijing, China; version 3.32; Whitehead Institute for Biomedical Research, Cambridge, MA, USA; http://www.broad.mit.edu/mpg/haploview/) [22] was used to determine frequencies of SNPs in the *Caspase-3* gene. Tag SNPs were selected using the web-based Tagger algorithm (http://www.broad.mit.edu/mpg/tagger/) with a minor allele frequency $\geq$ 0.05 and a linkage disequilibrium coefficient of 0.8.

Sample collection

Peripheral venous blood was collected from all participants in the early morning (07:00—08:00). Participants fasted for 10 hours prior to blood collection. After taking 2 mL of blood, we added sodium citrate for anticoagulation and preserved it at 4°C. Genomic DNA was extracted from whole blood samples using a Whole Blood Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) in accordance with the manufacturer's instructions. The purity and concentration of the DNA samples were measured by 1% agarose gel electrophoresis and spectrophotometer (Shanghai Mapada Instrument, Shanghai, China) analysis.

Polymerase chain reaction—restriction fragment length polymorphism detection

Polymerase chain reaction (PCR)—restriction fragment length polymorphism was used for the detection of *Caspase-3* gene polymorphisms. Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) was used for designing and verifying the PCR primers according to each locus of *Caspase-3*. The primers were synthesized by Shanghai Sangon Biotech (Shanghai, China) and the sequenced are presented in Table 1. The *Caspase-3* PCR reaction system (20 μL) included: 1 μL of both forward and reverse primers, 2.0 μL of template DNA, 7 μL of 2× Mix PCR buffer, and 7 μL of distilled water. PCR conditions were: 5 minutes of initial denaturation at 94°C, 30 seconds of denaturation at 94°C, 30 seconds of annealing at 58°C, and 30 seconds of extension at 72°C for 34 cycles, followed by a 5-minute extension at 72°C. The PCR product was subjected to RFLP testing. The whole reaction system (20 μL) contained 10 μL of PCR product, 12.5 μL of distilled water, 2 μL of enzyme digestion buffer and 0.5 μL of endonuclease and underwent 4 hours of endonuclease digestion at 37°C. The digested products were run on a 1.5% agarose gel, stained with ethidium bromide and observed and photographed under an ultraviolet gel imaging system.

Table 1 Primer sequences for *Caspase-3*.

Polymorphism	Primer sequence (5′—3′)
rs4647693	F: AAT TCT GTT GCC ACC TTT CG
	R: ACC TTC TGC GTG TTT GCT CT
rs4647610	F: AAC CGC TTC AAG AAA TCC TG
	R: ATC TGC CTT GTT GAG CCA CT
rs12108497	F: AGC CGC TCA CGC ATC ATA GT
	R: AGT CCG CAT CCA GCC AGG TA

Statistical analysis

Statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). A Chi-square test was applied to estimate whether the allele frequency distribution was in accordance with Hardy—Weinberg equilibrium. Measurement data were expressed as the mean ± standard deviation. The difference between the two groups was compared using *t* test. Enumeration data were detected by χ^2-test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the relative risk of LDH. The haplotype analysis was performed using SHEsis software (http://analysis.bio-x.cn/SHEsisMain.htm). Binary logistic regression analysis was used to identify whether the clinical factors were the risk factors for LDH. All tests were two-sided, and a significant difference was indicated when $p < 0.05$.

Results

Comparison of clinical data

As shown in Table 2, there were 107 patients in the case group, including 68 men and 39 women with a mean age of 38.75 ± 12.31 years. There were 121 individuals in the control group (71 men and 50 women with a mean age of 38.25 ± 11.73 years). There were no significant differences in age, sex, BMI, smoking history, or drinking history between the case and control groups (all $p > 0.05$). However, comparisons of the family history of LDH, amateur sports, leisure activities, bed types, and spine load grade between the case and control group revealed significant differences (all $p < 0.05$).

Genotype and allele frequency distribution of rs4647693, rs4647610, and rs12108497 SNPs in the case and control groups

The results of the Hardy—Weinberg Equilibrium showed that the genotype frequency distributions of *Caspase-3* SNPs rs4647693, rs4647610, and rs12108497 in the case and control groups were in line with genetic balance (all $p > 0.05$), which showed that the genotype frequency of the three SNPs were at equilibrium and were representative. Pairwise linkage disequilibrium in *Caspase-3* was examined using Haploview software (Broad Institute of MIT and Harvard, Cambridge, MA, USA). Linkage disequilibrium existed between rs4647610 and rs12108497 ($r^2 = 0.882$, D = 0.946; Table 3). As shown in Table 4, the genotype and allele frequencies of *Caspase-3* SNPs rs4647693, rs4647610, and rs12108497 in the case and control groups were significantly different (all $p < 0.05$). In the case group, the mutant genotype (GA), recessive models (GA+AA), and A allele might

Table 2 Comparison of clinical data in case and control groups.

	Case group (n = 107)	Control group (n = 121)	χ^2/t	p
Age (y)	38.75 ± 12.31	38.25 ± 11.73	0.314	0.754
Sex (male/female)	68/39	71/50	0.567	0.452
BMI (kg/m^2)	23.07 ± 2.95	22.96 ± 3.10	0.274	0.785
Family history of LDH (yes/no)	21/86	11/110	5.224	**0.022**
Smoking history (yes/no)	40/67	39/82	0.666	0.415
Drinking history (yes/no)	16/91	20/101	0.106	0.745
Amateur sports (yes/no)	24/83	45/76	5.862	**0.016**
Leisure activities (yes/no)	75/32	108/13	10.640	**0.001**
Bed types				
Soft bed	31	17		
Hard bed	76	104	7.608	**0.006**
Spine load grade				
1–2	48	95		
3–4	59	26	27.500	**< 0.001**

Data are presented as mean ± standard deviation or *n*.
BMI = body mass index; LDH = lumbar disc herniation.
Significant differences ($p < 0.05$) are noted in bold.

Table 3 The linkage disequilibrium parameters of TagSNPs in *Caspase-3*.

	rs4647610		rs12108497	
	D′	r^2	D′	r^2
rs4647693	0.790	0.563	0.608	0.465
rs4647610	—	—	0.946	0.882

be the protective factor for LDH (GA vs. GG: OR = 0.048, 95% CI = 0.011–0.206; GA+AA vs. GG: OR = 0.250, 95% CI = 0.250; and A vs. G: OR = 0.522, 95% CI = 0.294–0.922). Both mutant genotype (GG/AG) and G allele frequencies of rs4647610 were higher in the case group (all $p < 0.05$) compared to the control group, suggesting that the G allele may be the risk factor for LDH (AG vs. AA: OR = 3.254, 95% CI = 1.245–8.506; GG vs. AA: OR = 1.803, 95% CI = 1.007–3.229; AG+GG vs. AA: OR = 2.064, 95% CI = 1.205–3.533; and G vs. A: OR = 1.782, 95% CI = 1.210–2.625). The TT genotype of rs12108497, as well as the CC genotype and the C allele might increase the risk of LDH by 2.022-fold (95% CI = 1.110–3.682, $p < 0.05$) and 1.833-fold (95%CI = 1.234–2.722, $p < 0.05$).

Analysis of different *Caspase-3* SNPs and clinical data

No significant differences were found between the genotypes of *Caspase-3* SNPs rs4647693, rs4647610, and rs12108497 and age, sex, BMI, smoking history, drinking history, amateur sports, leisure activities, and bed types (all $p > 0.05$). Significant differences in family history of LDH and spine load grade were found between the polymorphic wild-type genotype and the mutant genotypes of rs4647610 and rs12108497 (all $p < 0.05$). Additionally, for the polymorphic wild-type genotype (GG) and mutant genotype (GA+AA) of rs4647693 in the case and control group, smoking was also significantly different ($p < 0.05$; Table 5).

Haplotype analysis

The haplotypes of three loci in the *Caspase-3* gene are shown in Table 6. We applied SHEsis software (http://analysis.bio-x.cn/SHEsisMain.htm) to analyze the haplotypes of the three loci of the participants from the case and control groups. Haplotype frequencies lower than 3% were excluded. The results showed that among the five haplotypes, the A-A-T (rs4647693-rs4647610-rs12108497) haplotype appeared to be a protective factor against LDH (OR = 0.521, 95% CI = 0.294–0.922, $p = 0.024$), whereas G-G-C appeared to be a risk factor for LDH (OR = 2.075, 95% CI = 1.364–3.156, $p < 0.001$).

Logistic regression analysis

Binary logistic regression analysis was performed to obtain an updated OR-Exp (B) with LDH as the dependent variable and family history of LDH, amateur sports, leisure activities, bed types, spine load grade, genotypes of *Caspase-3* (rs4647693 (GA+AA/GG), rs4647610 (AA/AG+GG), and rs12108497 (TC+CC/TT) as independent variables. The analysis showed that the GA+AA genotype of rs4647693 was a protective factor for the risk of LDH ($p < 0.05$), whereas spine load grade was an independent risk factor for LDH ($p < 0.05$; Table 7).

Discussion

LDH, one of the most typical musculoskeletal diseases, has various genetic and environmental factors, but the pathogenic mechanism of LDH is still unclear. In the present study, we conducted a controlled case study of three SNP sites in the *Caspase-3* gene, rs4647693, rs4647610, and rs12108497, to determine the relationship between the gene and LDH risk. Our study has shown that the genotype and allele frequencies of rs4647693, rs4647610, and rs12108497 in the *Caspase-3* gene were significantly different between the LDH patients and the controls, suggesting that *Caspase-3*

Table 4 The genotype frequency distributions of *Caspase-3* SNPs rs4647693, rs4647610, and rs12108497 in patient and control groups.

		Case group (n = 107)	Control group (n = 121)	χ^2	OR (95% CI)	p
rs4647693	GG	96	83			
	GA	2	36	12.230	0.048 (0.011–0.206)	**< 0.001**
	AA	9	2	3.330	0.257 (0.054–1.224)	0.068
	GA+AA	11	38	15.020	0.250 (0.120–0.521)	**< 0.001**
	G	194 (90.65)	202 (82.64)		Ref	
	A	20 (9.35)	40 (17.36)	5.128	0.522 (0.294–0.922)	**0.024**
rs4647610	AA	54	82			
	AG	15	7	6.242	3.254 (1.245–8.506)	**0.013**
	GG	38	32	3.975	1.803 (1.007–3.229)	**0.046**
	AG+GG	53	39	7.062	2.064 (1.205–3.533)	**0.008**
	A	123 (57.48)	171 (70.66)		Ref	
	G	91 (42.52)	71 (29.34)	8.619	1.782 (1.210–2.625)	**0.003**
rs12108497	TT	60	83			
	TC	9	12	0.006	1.038 (0.411–2.619)	0.938
	CC	38	26	5.380	2.022 (1.110–3.682)	**0.020**
	TC+CC	47	38	3.807	1.711 (0.996–2.941)	0.051
	T	129 (60.28)	178 (73.55)		Ref	
	C	85 (39.72)	64 (26.45)	9.096	1.833 (1.234–2.722)	**0.003**

CI = confidence interval; OR = odds ratio.
Significant differences ($p < 0.05$) are noted in bold.

Table 5 Analysis of different genotypes and clinical data.

	n	rs4647693		p	rs4647610		p	rs12108497		p
		GA+AA	GG		AG+GG	AA		TC+CC	TT	
		(n = 11)	(n = 96)		(n = 53)	(n = 54)		(n = 47)	(n = 60)	
Age (y)		40.00 ± 11.28	38.60 ± 12.47	0.724	37.66 ± 11.87	39.815 ± 12.75	0.368	37.68 ± 11.85	39.58 ± 12.70	0.430
Sex										
Male	68	9	59		33	35		30	38	
Female	39	2	37	0.184	20	19	0.784	17	22	0.958
BMI (kg/m^2)		23.36 ± 3.44	23.04 ± 2.91	0.730	22.92 ± 3.08	23.22 ± 2.83	0.604	22.71 ± 3.10	23.35 ± 2.82	0.268
Family history of LDH										
Yes	21	2	19		15	6		14	7	
No	86	9	77	0.899	38	48	**0.025**	33	53	**0.019**
Smoking history										
Yes	40	5	35		21	19		18	22	
No	67	6	61	0.559	32	35	0.635	29	38	0.863
Drinking history										
Yes	16	0	16		10	6		10	6	
No	91	11	80	0.142	43	48	0.261	37	54	0.105
Amateur sports										
Yes	24	5	19		13	11		12	12	
No	83	6	77	0.053	40	43	0.606	35	48	0.496
Leisure activities										
Yes	75	10	65		35	40		31	44	
No	32	1	31	0.111	18	14	0.364	16	16	0.408
Bed types										
Soft bed	31	3	28		18	13		18	13	
Hard bed	76	8	68	0.896	35	41	0.260	29	47	0.060
Spine load grade										
1–2	48	10	38		15	33		14	34	
3–4	59	1	58	**0.001**	38	21	**< 0.001**	33	26	**0.006**

Data are presented as mean ± standard deviation or n.
BMI = body mass index; LDH = lumbar disc herniation.
Significant differences ($p < 0.05$) are noted in bold.

Table 6 Haplotype frequencies of rs4647693, rs4647610 and rs12108497 in *Caspase-3* gene in both the case and the control.

Haplotype (rs4647693-rs4647610-rs12108497)	Case (freq)	Control (freq)	χ^2	Fisher *p*	OR (95%CI)
AAT	20 (0.093)	40 (0.165)	2.152	**0.024**	0.521 (0.294—0.922)
GAC	10 (0.047)	14 (0.058)	0.290	0.590	0.796 (0.347—1.828)
GAT	93 (0.434)	117 (0.483)	1.085	0.298	0.822 (0.568—1.189)
GGC	75 (0.350)	50 (0.206)	11.851	**< 0.001**	2.075 (1.364—3.156)
GGT	16 (0.075)	21 (0.087)	0.226	0.063	0.849 (0.431—1.670)

CI = confidence interval; OR = odds ratio.
Significant differences ($p < 0.05$) are noted in bold.

Table 7 Binary logistic regression analysis for investigating the association between lumbar disc herniation risk and *Caspase-3* gene polymorphisms.

Independent variable	B	SE	Wald	df	*p*	Exp (B)	95% CI for Exp (B)	
							Lower	Upper
Family history of lumbar disc herniation	−0.800	0.528	2.297	1	0.130	0.449	0.160	1.264
Amateur sports	0.577	0.369	2.445	1	0.118	1.781	0.864	3.671
Leisure activities	0.790	0.458	2.980	1	0.084	2.203	0.899	5.402
Bed types	−0.895	0.466	3.691	1	0.055	0.409	0.164	1.018
Spine load grade	−1.756	0.410	18.348	1	**< 0.001**	0.173	0.077	0.386
rs4647693	−3.064	0.604	25.764	1	**< 0.001**	0.047	0.014	0.153
rs4647610	−0.072	0.459	0.024	1	0.876	0.931	0.379	2.288
rs12108497	0.118	0.463	0.064	1	0.800	1.125	0.454	2.789

B = partial regression coefficient; df = degree of freedom; Exp (B) = odds ratio; SE = standard error; Wald = Wald χ^2.
Significant differences ($p < 0.05$) are noted in bold.

gene polymorphisms might be used as genetic determinants for LDH susceptibility. In particular, our findings provide a quantitative basis for the three SNPs in the *Caspase-3* gene as a risk factor for or a protective factor against the susceptibility of LDH. For instance, the *Caspase-3* rs4647693 SNP, which was identified as a protective factor, was related to a decreased risk of LDH; whereas rs4647610 and rs12108497 SNPs were both associated with a significantly elevated risk of disc degeneration, suggesting that these *Caspase-3* variants are likely to be susceptibility markers for LDH. These results could be due to the fact that the genetic polymorphisms of rs4647610 and rs12108497 may affect LDH susceptibility by changing the transcription efficiency and function of the *Caspase-3* gene, which is considered as a major player in the apoptotic process [15]. To some extent, apoptosis may have an important role in maintaining normal disk function by eliminating aberrant cells [18]. As discussed above, the impaired activity of *Caspase-3* might be connected to LDH risk [23].

In addition, we found statistically significant differences in family history of LDH, amateur sports, leisure activities, bed types, and lumbar load grade between the two study groups. By contrast, no significant differences were observed regarding age, sex, BMI, smoking, or drinking, which made it possible for us to analyze further the interaction between the environmental factors and *Caspase-3*.

Because many complex diseases are caused by the interaction between genetic and environmental factors, we also examined a possible genetic association between the genotypes and environmental risk factors, including smoking, bed type, amateur sports, and leisure activities. According to our study, all three SNPs in *Caspase-3* had an association with spine load grade and high lumbar load grade, which contribute significantly to the risk of LDH. Comparable results were reported in previous studies, which highlighted the pivotal role of lumbar load in leading to excessive apoptosis of disk cells and structural changes in intervertebral discs, thus playing an etiologic role in the development of lumbar disc narrowing, as well as LDH [24—26]. In addition, family history of LDH showed a significantly increased correlation with *Caspase-3* rs4647693 genotypes among patients, which might strongly confer individual LDH susceptibility. Furthermore, previous family and twin studies have documented the substantial heritability of LDH and support the notion that familial susceptibility is a pathogenic factor to lumbar degeneration, which could elevate LDH risk [27,28].

Our findings of the relation between the three SNPs in *Caspase-3* and the risk of LDH further strengthen the findings from the single-locus analyses, which revealed that haplotype G-G-C (rs4647693-rs4647610-rs12108497) of these three variants was also associated with increased risk of LDH, whereas A-A-T haplotype was associated with a reduced occurrence of LDH. These findings suggest that the development of LDH could be influenced by SNP, as well as haplotype differences. Additionally, logistic regression analysis showed that both spine load grade and the *Caspase-3* rs4647693 polymorphism were closely related with LDH and were independent risk factors for LDH.

As for the present study, some limitations that may bias our analyses should be taken into consideration. First of all, the studied group of patients is too small to make arbitrary

conclusions regarding genetic polymorphisms in the *Caspase-3* gene. Therefore, a larger and well-designed analysis based of different populations is required to elucidate fully the relevant mechanisms in future research. Second, all of the study participants were matched for sex and geographical area, which would have reduced the effect of population stratification, although it cannot be fully excluded. Furthermore, we will continue to explore other gene polymorphisms and combinations of polymorphisms to search for the potential causes of LDH.

Conclusion

Our study provides strong evidence that *Caspase-3* rs4647693, rs4647610, and rs12108497 SNPs are tightly linked to genetic susceptibility to LDH, and their haplotypes are greatly associated with the risk of LDH. Furthermore, the association between *Caspase-3* genetic polymorphisms and LDH risk also appears to be influenced by spine load grade and family history. Therefore, these findings may lead to a better understanding of the pathogenic mechanisms of LDH and suggest promising targets for treatment and novel therapeutic strategies for LDH.

References

[1] Han L, Zhao P, Guo W, Wei J, Wang F, Fan Y, et al. Short-term study on risk-benefit outcomes of two spinal manipulative therapies in the treatment of acute radiculopathy caused by lumbar disc herniation: study protocol for a randomized controlled trial. Trials 2015;16:122.

[2] Rut M, Machoy-Mokrzynska A, Reclawowicz D, Sloniewski P, Kurzawski M, Drozdzik M, et al. Influence of variation in the catechol-O-methyltransferase gene on the clinical outcome after lumbar spine surgery for one-level symptomatic disc disease: a report on 176 cases. Acta Neurochir (Wien) 2014;156:245—52.

[3] Li YF, Tang XZ, Liang CG, Hui YM, Ji YH, Xu W, et al. Role of growth differentiation factor-5 and bone morphogenetic protein type II receptor in the development of lumbar intervertebral disc degeneration. Int J Clin Exp Pathol 2015;8:719—26.

[4] Mu J, Ge W, Zuo X, Chen Y, Huang C. A SNP in the 5′UTR of GDF5 is associated with susceptibility to symptomatic lumbar disc herniation in the Chinese Han population. Eur Spine J 2014;23:498—503.

[5] Lin WP, Wang XJ, Wang CR, Zhang LQ, Li N, Wang FS, et al. Polymorphism in the hypoxia-inducible factor 1alpha gene may confer susceptibility to LDD in Chinese cohort. PLoS One 2013;8:e73158.

[6] Suzuki S, Fujita N, Hosogane N, Watanabe K, Ishii K, Toyama Y, et al. Excessive reactive oxygen species are therapeutic targets for intervertebral disc degeneration. Arthritis Res Ther 2015;17:316.

[7] Sansoni V, Perego S, Colombini A, Banfi G, Brayda-Bruno M, Lombardi G. Interplay between low plasma RANKL and VDR-FokI polymorphism in lumbar disc herniation independently from age, body mass, and environmental factors: a case-control study in the Italian population. Eur Spine J 2016;25:192—9.

[8] Cong L, Zhu Y, Tu G. Association between the expression of aggrecan and the distribution of aggrecan gene variable number of tandem repeats with symptomatic lumbar disc herniation. Zhonghua Wai Ke Za Zhi 2015;53:116—20.

[9] Sedighi M, Haghnegahdar A. Role of vitamin D3 in treatment of lumbar disc herniation—pain and sensory aspects: study protocol for a randomized controlled trial. Trials 2014;15:373.

[10] Cong L, Zhu Y, Pang H, Guanjun TU. The interaction between aggrecan gene VNTR polymorphism and obesity in predicting incident symptomatic lumbar disc herniation. Connect Tissue Res 2014;55:384—90.

[11] Hong Q, Yu S, Yang Y, Liu G, Shao Z. A polymorphism in JMJD2C alters the cleavage by caspase-3 and the prognosis of human breast cancer. Oncotarget 2014;5:4779—87.

[12] Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol 2013;14:32.

[13] Xing Y, Wang H, Liu X, Yu X, Chen R, Wang C, et al. Meta-analysis of the relationship between single nucleotide polymorphism rs72689236 of caspase-3 and Kawasaki disease. Mol Biol Rep 2014;41:6377—81.

[14] Kuo HC, Yu HR, Juo SH, Yang KD, Wang YS, Liang CD, et al. CASP3 gene single-nucleotide polymorphism (rs72689236) and Kawasaki disease in Taiwanese children. J Hum Genet 2011;56:161—5.

[15] Li B, Liu H, Gong F, Sun P, Yan Y, Jia B. Molecular epidemiologic correlation analysis between caspase3 gene polymorphism and gastric cancer susceptibility. Cell Biochem Biophys 2014;70:1647—53.

[16] Liu X, He Y, Li F, Huang Q, Kato TA, Hall RP, et al. Caspase-3 promotes genetic instability and carcinogenesis. Mol Cell 2015;58:284—96.

[17] Xu HL, Xu WH, Cai Q, Feng M, Long J, Zheng W, et al. Polymorphisms and haplotypes in the caspase-3, caspase-7, and caspase-8 genes and risk for endometrial cancer: a population-based, case-control study in a Chinese population. Cancer Epidemiol Biomarkers Prev 2009;18:2114—22.

[18] Sudo H, Minami A. Caspase 3 as a therapeutic target for regulation of intervertebral disc degeneration in rabbits. Arthritis Rheum 2011;63:1648—57.

[19] Reddi AH. Inhibition of cell death in the intervertebral disc by caspase 3 small interfering RNA. Arthritis Rheum 2011;63:1477—8.

[20] Ponzer S, Skoog A, Bergström G. The Short Musculoskeletal Function Assessment Questionnaire (SMFA): cross-cultural adaptation, validity, reliability and responsiveness of the Swedish SMFA (SMFA-Swe). Acta Orthop Scand 2003;74:756—63.

[21] Battié MC, Videman T, Gibbons LE, Fisher LD, Manninen H, Gill K. 1995 Volvo Award in clinical sciences. Determinants of lumbar disc degeneration. A study relating lifetime exposures and magnetic resonance imaging findings in identical twins. Spine (Phila Pa 1976) 1995;20:2601—12.

[22] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263—5.

[23] Yamada K, Sudo H, Iwasaki K, Sasaki N, Higashi H, Kameda Y, et al. Caspase 3 silencing inhibits biomechanical overload-induced intervertebral disk degeneration. Am J Pathol 2014;184:753—64.

[24] Zhang YG, Zhang F, Sun Z, Guo W, Liu J, Liu M, et al. A controlled case study of the relationship between environmental risk factors and apoptotic gene polymorphism and lumbar disc herniation. Am J Pathol 2013;182:56—63.

[25] Ahsan MK, Matin T, Ali MI, Ali MY, Awwal MA, Sakeb N. Relationship between physical work load and lumbar disc herniation. Mymensingh Med J 2013;22:533—40.

[26] Sun Z, Ling M, Chang Y, Huo Y, Yang G, Ji Y, et al. Single-nucleotide gene polymorphisms involving cell death pathways: a study of Chinese patients with lumbar disc herniation. Connect Tissue Res 2013;54:55—61.

[27] Sun ZM, Ling M, Chang YH, Liu ZZ, Xu HH, Gong LQ, et al. Case—control study of the risk factors of lumbar intervertebral disc herniation in 5 northern provinces of China. Nan Fang Yi Ke Da Xue Xue Bao 2010;30:2488—91.

[28] Zawilla NH, Darweesh H, Mansour N, Helal S, Taha FM, Awadallah M, et al. Matrix metalloproteinase-3, vitamin D receptor gene polymorphisms, and occupational risk factors in lumbar disc degeneration. J Occup Rehabil 2014;24:370—81.